Child Health Research Project

Synopsis: *Vibrio cholerae* O139 - Detection, Characterization, and Control

October 1998 Number 4

Cholera is an acute diarrheal illness caused by infection of the intestine with the bacterium *Vibrio cholerae*, and is characterized by profuse watery diarrhea, vomiting, and leg cramps. Rapid loss of body fluids leads to dehydration and shock, and without treatment, death can occur within hours. As of September 30, 1998, the World Health Organization estimates that there have been 198,436 cases of cholera worldwide, a dramatic rise in reported cases since last year. It must be noted, however, that many countries are reluctant to report cholera cases because of the possible impact on the economy, and as a result only about 10% of cholera cases are ever documented. Further, many cholera endemic countries in Asia with incidence rates estimated to be about 1 per 1000 do not report cholera at all. The El Niño phenomenon, with its related heavy rainfalls and floods, may be responsible for the increase in the number of cases and deaths. This global increase in cholera is an important concern for ministries of health, NGOs, and Missions administering health research and programs in affected areas. All agencies should consider strengthening cholera preparedness activities including keeping stocks of medicine in reserve, increasing training and health education activities, and setting up cholera task forces in advance of possible epidemic spread.

The worldwide spread of cholera began in 1817, and by 1823 the first pandemic of cholera had spread from the Ganges River delta to much of Asia and Africa. During the 19th century, cholera repeatedly spread along routes of trade and travel from India to Europe, Africa, and North America. Five periods of pandemic spread occurred before 1900: from 1817 to 1823; 1826 to 1837; 1846 to 1862; 1864 to 1875; and 1887 to 1896. In each country involved, thousands were affected, with case-fatality rates often approaching 50%. The sixth pandemic (1902 to 1923) also involved severe epidemics, especially in Asia, but outbreaks in Africa and Europe were more limited than in previous pandemics and the Western Hemisphere was not involved. The sixth pandemic and presumably the previous pandemics were due to the classic biotype of *V. cholerae* O1. This biotype decreased in frequency in the 1960s and has largely disappeared except in Bangladesh, where it reemerged in epidemic form in 1982.(1)

The seventh pandemic, which continues today, is generally considered to have started in 1961. The causative agent of this pandemic was first isolated in 1905 by Gotschlick from pilgrims returning from Mecca at the El Tor quarantine camp in Egypt. Although this organism was initially considered nonpathogenic, outbreaks of severe disease between 1937 and 1958 confirmed its ability to cause epidemics.(2) An outbreak caused by *V. cholerae* O1 biotype El Tor in Sulawesi in 1961 was the beginning of the seventh pandemic. From there it quickly spread to Java, Sarawak, Borneo, the Philippines, and most of Southeast Asia. Between 1963 and 1969, this organism continued its spread across the Asian mainland. The El Tor biotype eventually replaced classic *V. cholerae* in Asia. In 1970, the pandemic continued its westward progression, involving the Middle East and the Soviet Union, and resulting in serious outbreaks in Spain, Portugal, and Italy. Since then, nearly all countries in Africa have been involved with cholera outbreaks, and in 1991, the epidemic spread to Latin America.

Detection of Cholera O139 in Bangladesh

In October 1992, a new strain of epidemic cholera was detected in Madras, India, and rapidly spread through the Indian subcontinent.(3) In January 1993, cholera O139 made its first appearance in Bangladesh at a gathering of Muslim pilgrims near Dhaka.(4) As in the Indian epidemic, most of the victims were adults – indicating that the population had no previous exposure to the strain. All patients displayed the usual cholera symptoms – severe diarrhea (4 to 12 ml/kg body weight/hr), vomiting, and dehydration – and responded to standard treatment protocols of intravenous and oral rehydration. By February, scientists at the ICDDR,B: Centre for Health and Population Research in Dhaka recognized that the "non-O1" serotype

Figure 1: Number of Patients with Cholera in Matlab, Bangladesh 1993–94

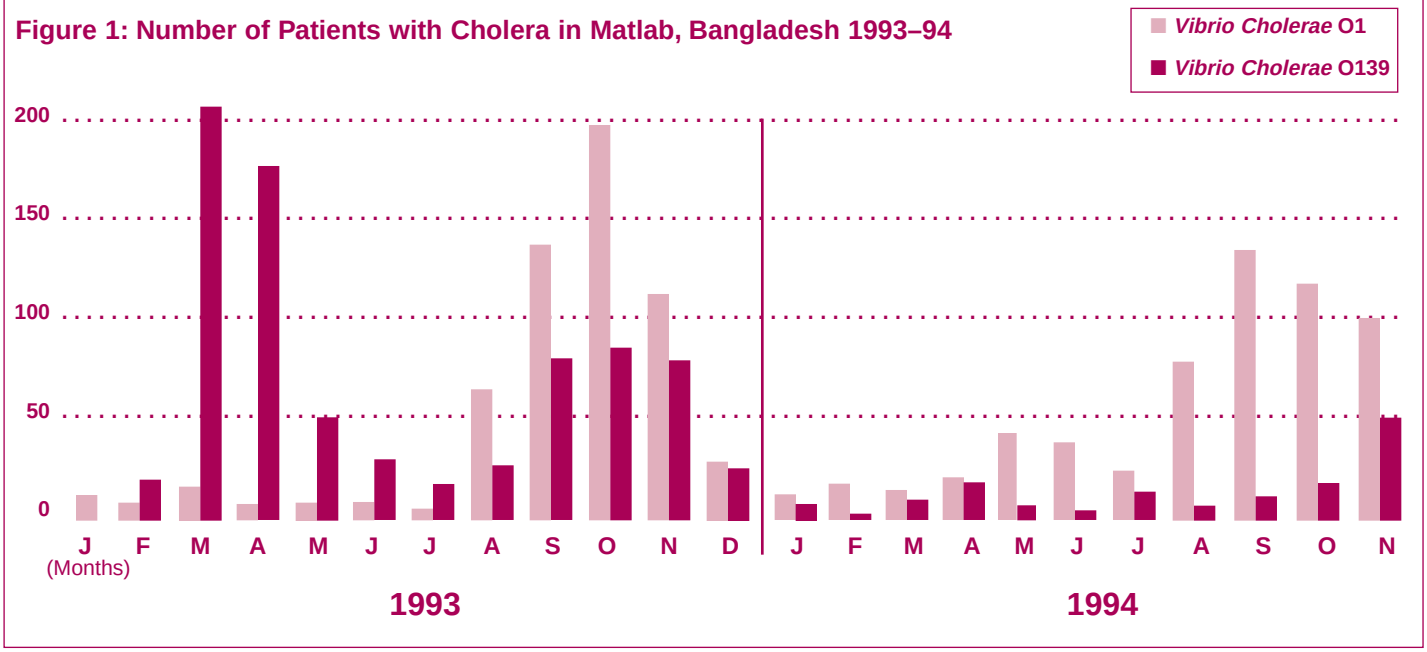

Figure 2: The Spread of Cholera 0139

causing disease in their hospital was the same as the O139 serogroup that had been recently defined in the laboratory of Dr. Shimada in Japan.[5,6] A diagnostic antiserum was quickly prepared and distributed to hospitals involved in cholera diagnosis throughout the region. In the spring and summer of that year, O139 disease far outnumbered cases of classic O1 cholera, but by the fall, cases of O139 were on the wane (Figure 1). At year's end 170,000 cases of O139 cholera, which resulted in only 2,000 deaths, were documented in Bangladesh, and the O139 serotype was detected in Thailand, Malaysia, Nepal, Pakistan, Burma (Myanmar), and China (Figure 2).[7,8] Several cases of traveler's O139 cholera were also noted in the United States, United Kingdom, Japan, Korea, Hong Kong, and Singapore.[9] In 1994 there were fewer cases of O139 cholera than of the O1 serotype, and the O139 epidemic was essentially over by the end of that year. In 1995, however, a single case of O139 cholera of uncertain epidemiology was noted in Denmark,[10] indicating that the disease may have already spread to Europe.

Characterization of Cholera O139

The ICDDR,B has continued to examine the characterization and natural history of *Vibrio cholerae* O139. Researchers there have documented the many similarities in morphology to the O1 El Tor biotype (Table 1), leading many to postulate that O139 evolved from El Tor in the Bay of Bengal. Like the O1 serogroup, O139 has also been found in association with plankton in pond surface waters.[11] Work done at the ICDDR,B in collaboration with the Johns Hopkins School of Public Health and the University of Maryland has further elucidated that the plankton serve as a reservoir for *V. cholerae* in the aquatic environment. Important support for this hypothesis was found in the timing of plankton blooms and cholera outbreaks in Bangladesh. Outbreaks of disease almost always follow the zooplankton bloom in September and October. A second and lesser peak of cholera cases usually occurs after the summer phytoplankton bloom in early summer.

One of the most important differences between O139 and El Tor is the former's possession of a thin capsular layer which increases the virulence (especially of bacteriemia due to serum resistance) and invasive properties of the strain, and allows it to survive for extended periods of time in surface water. Another difference between the two strains is the composition of the lipopolysaccharide (LPS) in the capsule, which has allowed for the development of two rapid diagnostic tests using monoclonal antibodies to the LPS in O139. The first test, or coaglutination test (COAT),[12] has a 92% sensitivity and 100% specificity when compared to culture as the gold standard, with 100% positive and 95% negative predictive values. The second test – the Bengal SMART (Sensitive Membrane Antigen Rapid Test) [13] – has a sensitivity of 100% and a specificity of 97% and takes only 15 minutes to complete. The speed and accuracy of diagnosis make SMART suitable for use in the field for rapid detection of *V. cholerae* O139.

Because the use of antimicrobials is an important therapeutic adjunct to cholera treatment – and reduces the duration of the illness – documentation of resistance patterns have also formed an important part of continuing work at the ICDDR,B. Early in the epidemic, *V. cholerae* O139 isolates from Bangladesh and India were reported to be susceptible to tetracycline, ampicillin, chloramphenicol, erythromycin, and ciprofloxacin but resistant to cotrimox-

Table 1 Similarities between *V. cholerae* O1 El Tor and *V. cholerae* O139

Morphology
Culture
Physiology and sugar fermentation
Fimbrial antigens
Cholera toxin (ctx), gene sequences for zona occludens
Toxin and accessory cholera enterotoxin
In vitro invasiveness for Hep-2 cells
Possession of (ctx) element and its location in the "core" region of the chromosome
Possession and location of toxin coregulated pili gene (tcpA) and iron-regulated genes (irgA, viu, and fur) on similar sites in the chromosomes
Outer membrane protein profiles
Sequence structure for ctxAB and 16SrRNA gene
Regulation of virulence genes by toxR
Multilocus enzyme electropherotype
Ribotype
Ctx genotype
Pulsed-field gel electropherotype

azole and streptomycin.[5] More recent data from 173 isolates collected in 1992 – 93 from Bangladesh, India, and Thailand showed that most isolates were susceptible to tetracycline and the newer quinolones (norfloxacin, ofloxacin, tosufloxacin, ciprofloxacin, and sparfloxacin), while 95% of the isolates were resistant to chloramphenicol, furazolidone, streptomycin, sulfamethoxazole, and trimethoprim. Six isolates were resistant to ampicillin, tetracycline, chloramphenicol, kanamycin, gentamicin, sulfamethoxazole, and trimethoprim, with the resistance encoded by a 200-kb plasmid in all six strains.[14]

As soon as it became clear that *V. cholerae* O139 was capable of both causing epidemic cholera and spreading rapidly to nearby countries, vaccine development began. Because both killed and live, attenuated oral vaccines had already been developed against *V. cholerae* O1, the initial strategy was to incorporate the new O139 antigens into the O1 vaccines.[15] Live vaccine prototypes were constructed by deleting genes for specific virulence determinants (cholera toxin, zona occludens toxin, and accessory cholera enterotoxin) and reintroducing genes responsible for the production of the B subunit of the cholera enterotoxin. One of the live vaccines (CVD 112) was safe when administered to six human volunteers and had an 84% short-term protective efficacy after challenge. [16] Volunteer studies at Johns Hopkins School of Public Health demonstrated the vaccine strain Bengal-15 to be safe and immunigenic. More recent work at the ICDDR,B testing an oral bivalent B O1/O139 whole cell vaccine in 20 adults showed the vaccine to be safe and effective in the production of vibriocidal antibodies, antibody-secreting cell responses in blood and gut, and plasma and fecal antibodies to vaccine components.[17]

Current Concerns

In August 1996, *V. cholerae* O139 reemerged in Southern India, in Calcutta, Vellore, and Madras, causing severe illness in those infected. Upon laboratory analysis, however, the reemerged organism was sensitive to cotrimoxazole and the vibriostatic agent pteridine – unlike the strains tested in 1993 – 94.[18] A more comprehensive examination of the phenotypic and genotypic changes in O139 isolates from 1993 to 1996 demonstrated the evolution of four different genotypes.[19] All isolates possessed cholera toxin (ctx) genes, and genes for type-A toxin coregulated pili (tcpA), hemagglutinin/protease, and a capsule. This indicates that the genes responsible for virulence and the genes encoding for the surface organelles (fimbriae) required for intestinal colonization were intact. Further, all early isolates were cAMP hemolysin positive and resistant to the vibriostatic compound O/129, while all of the 1996 isolates were negative and susceptible. During the 1993 epidemic, these properties were used for the presumptive diagnosis of cholera O139. The recent changes in phenotyping described above suggest that they should no longer be used for this purpose.

The emergence of a new clone of *V. cholerae* O1 El Tor to replace O139 is also an issue of current research. 50 El Tor vibrios from Asia and Africa from a period prior to the emergence of O139 were analyzed and compared with 32 isolates of El Tor from 1994 and beyond.[20] Genetic analysis for conserved rRNA, cholera toxin, and zona occludens toxin or in DNA sequences flanking the genes showed that the El Tor strains isolated before the emergence of O139 belonged to four different ribotypes and four different ctx genotypes. By comparison, all the isolates analyzed from after 1994 represent a new clone of El Tor vibrio distinctly different from the earlier clones of the El Tor vibrios that were replaced by the O139 vibrios. Although the level of cholera toxin production in the new El Tor clone is comparable to that of previous epidemic isolates, further analysis is required to assess its epidemic potential.

The Eighth Pandemic ?

V. cholerae O139 was first discovered as an epidemic organism which over the course of two years caused country-wide outbreaks in India and Bangladesh and spread to at least six other neighboring countries. This rapid spread is due to the lack of protective immunity in the populations it invaded and the presence of new, adaptive mechanisms that allow it to survive and multiply well in surface waters. Coupled with these changes is the current lack of adequate vaccine coverage against disease. In 1997, the Russian Ministry of Health announced that it had isolated *V. cholerae* O139 in Moscow sewage, and in 1998 the disease has been detected in India, Bangladesh, Thailand, Pakistan and possibly Afganistan. The fear that this new strain could become a global threat is still present, particularly in Asia, where extremely heavy rainfalls and floods have contributed to an increase in deaths due to cholera. Thus, efforts to monitor the spread of *V. cholerae* O139 at a global level must be strengthened, and research for an effective and safe vaccine continued.

References

1. Samadi AR, Huq MI, Shahid N, et al: Classical *Vibrio cholerae* biotype displaces El Tor in Bangladesh. 1983, *The Lancet* 1:805.

2. Glass R, Black RE: Epidemiology of cholera. In: *Cholera*, Barna D, Greenough WB III (eds). New York: Plenum Medical Book Co., p 129-154, 1992.

3. Ramamurthy T, Garg S, Sharma R, Bhattacharya SK, Nair GB, Shimada T, et al. Emergence of a novel strain of Vibrio cholerae with epidemic potential in southern and eastern India. 1993, *The Lancet* 341:703.

4. Shimada T, Nair GB, Deb BS, Albert MJ, Sack RB, Takeda Y. Outbreak of *Vibrio cholerae* non-O1 in India and Bangladesh. 1993, *The Lancet* 341:1347.

5. Cholera Working Group. Large epidemic of cholera-like disease in Bangladesh caused by *Vibrio cholerae* O139 synonym Bengal. 1993, *The Lancet* 342:387.

6. Islam MS, Hasan MK, Miah MA, Qadri F, Yunus M, Sack RB, Albert MJ. Isolation of *Vibrio cholerae* O139 Bengal from water in Bangladesh. 1993, *The Lancet* 342:430.

7. Chongsa-nguan M, Chaicumpa W, Moolasart P, Kandhasingha P, Shimada T, Kurazono H, Takeda Y. *Vibrio cholerae* O139 Bengal in Bangkok. 1993, *The Lancet* 342:430.

8. Fisher-Hoch S, Khan A, Inam-ul-Haq; Khan M. Ata; Mintz ED. *Vibrio cholerae* O139 in Karachi, Pakistan. 1993, *The Lancet* 342:1422.

9. Cheasty T, Said B, Rowe B, Frost J. *Vibrio cholerae* Serogroup O139 in England and Wales. 1993, *British Medical Journal* 307:1007.

10. Dalsgaard A, Nielsen GL, Echeverria P, Larsen JL, Schonheyder HC. *Vibrio cholerae* O139 in Denmark. 1995, *The Lancet*, 345:1537.

11. Huq A, Colwell RR, Chowdhury MAR, Xu B, Moniruzzaman IMS, Yunus M, Albert MJ. Coexistence of *Vibrio cholerae* O1 and O139 Bengal in plankton in Bangladesh. 1995, *The Lancet* 345:1249.

12. Quadri F, Chowdhury A, Hossain J, Chowdhury K, Azim T, Shimada T, Islam KM, Sack RB, Albert MJ. Development and evaluation of rapid monoclonal antibody-based coagglutination test for direct detection of *Vibrio cholerae* O139 synonym Bengal in stool samples. 1994, *J. Clin Microbiol* 32(6):1589.

13. Evaluation of the monoclonal antibody-based kit Bengal SMART for rapid detection of *Vibrio cholerae* O139 synonym Bengal in stool samples. 1995, *J. Clin. Mircrobiol* 33(3):732.

14. Yamamoto T, Nair GB, Albert MJ et al. Survey of in vitro susceptibilities of *Vibrio cholerae* O1 and O139 to antimicrobial agents. 1995, *Antimicrob Agents Chemother* 39:241.

15. Sack RB, Albert MJ, Siddique AK. Emergence of *Vibrio cholerae* O139. 1996, *Curr Clin Topics Infect Dis* 16:172.

16. Tacket CO, Losonsky G, Nataro JP, Comstock L, Michalski J, Edelman R, Kaper JB, Levine MM. Initial clinical studies of CVD 112 *Vibrio cholerae* O139 live oral vaccine: Safety and efficacy against experimental challenge. 1995, *J. Infect Dis* 172(3):883.

17. *Annual Report 1997*: ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh, May 1998.

18. Mitra R, Basu A, Dutta D, Nair GB, Takeda Y. Resurgence of *Vibrio cholerae* O139 Bengal with altered antibiogram in Calcutta, India. 1996, *The Lancet* 348:1181.

19. Albert MJ, Bhuiyan NA, Talukder KA, Faruque AS, Nahar S, Faruque SM, Ansaruzzaman M, Rahman M. Phenotypic and genotypic changes in *Vibrio cholerae* O139 Bengal. 1997, *J Clin Microbiol* 35:2588.

20. Faruque SM, Ahmed KM, Abdul A AR, Qadri F, Siddique AK, and Albert MJ. Emergence of a new clone of toxigenic *Vibrio cholerae* O1 biotype El Tor displacing *V. cholerae* O139 Bengal in Bangladesh. 1997, *J. Clin Microbiol* 35:624

Synopsis is published by the Child Health Research Project. For information, comments, or more copies of this issue please contact Laura Kelley (410) 614-5439, fax (410) 955-7159, e-mail: LKELLEY@JHSPH.EDU, or visit our website at http://ih1.sph.jhu.edu/chr/chr.htm. *Synopsis* Number 5, available in January 1999, will feature the Invasive Bacterial Infections Surveillance (IBIS) project.

The Child Health Research Project is a project of the United States Agency for International Development, and represents cooperative agreements between USAID and WHO, Harvard University, the ICDDR,B:Centre for Health and Population Research in Dhaka, Bangladesh, INCLEN: International Clinical Epidemiology Network, and Johns Hopkins School of Public Health.

Child Health Research Project

Child Health Research Project USAID
G/PHN/HN/CS 3.07-070
3rd Flr Ronald Reagan Building
Washington DC 20523-37
Attn: Dr. Ruth Frischer